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# Kinetics for Describing the Creaming of Protein-Stabilized O/W Emulsions by Multiple Light Scattering

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**Abstract:** In the present work, new kinetics to describe the creaming stability of oil-in-water emulsions determined by backscattering measurements (*BS*) is proposed. The emulsions assayed exhibited a different backscattering profiles regarding creaming destabilization, hyperbolic and sigmoid one. Hyperbolic behavior can be described by a second order kinetics, where  $k_h$  could be equaled to a rate constant that describes the creaming process and its values would indicate the stability of emulsions. While for the sigmoid *BS* pattern, kinetics with two terms, is adequate to describe the creaming process in contrast to kinetics previously reported in the literature. The  $k_h$  value has the same meaning as before, and  $k_s$  indicates the delaying effect on the creaming rate.

**Key words:** Emulsion, proteins, creaming, kinetic, multiple light scattering.

## 1. Introduction

When an emulsion is stored, physical characteristics such as color and viscosity change as a function of storage time and conditions. In the particular case of food emulsions, such changes must be controlled to ensure that emulsion characteristics remain within a given range of values, out of which the use or trade of such products is no longer possible in food. The two immiscible phases are generally an aqueous and a fatty one, the last being the dispersed one in most cases [1]. As soon as an emulsion has been formed by dispersion of one phase into the other, several physical processes begin simultaneously, which affect its kinetic stability. The destabilization process tends to reduce the interfacial surface and finally leads to the partial or total separation of the two immiscible phases [1]. The main mechanisms that contribute to destabilization are

creaming, flocculation and coalescence [1, 2]. The study of emulsion stability includes the analysis and quantification of changes due to these different destabilization mechanisms, and is essential for designing food emulsions. Creaming, which is a reversible process, results from differences in density between oil and aqueous phases; oil droplets, being less dense than water, tend to move to the upper portion of the emulsion. The creaming process has been analyzed using several different methods, including turbidimetric [3], gravimetric [4], volumetric [5-7] and conductimetric ones [8].

Devices that allow such analyses by the technique of multiple light scattering have been developed in the last years [9]. Several researchers have already used this technique to study the creaming stability [10-15].

Establishing kinetics describing the stability of emulsions in terms of creaming stability is of utmost importance. Kinetics of this kind has been already

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proposed by different authors [5, 16].

The main objective of the present work was to propose new kinetics to describe the creaming stability of oil-in-water emulsions determined by a technique of multiple light scattering. This kinetics with two terms, one of sigmoidal shape and one of second order, would better describe the creaming process than those previously reported by other authors.

## 2. Materials and Methods

### 2.1 Preparation of Soybean Protein Isolates (SPI)

The native SPI used in the present study was obtained by aqueous solubilization of defatted soy flour in alkaline solution (pH 8.0), followed by isoelectric precipitation, neutralization of the precipitate and freeze-drying, according to Petrucci and Anon [17]. A dispersion of SPI (30 mg/mL) in sodium phosphate buffer (0.01 M, pH 8.0) was prepared. A portion of the dispersion was frozen (SPI-F) and other portion was adjusted to pH 2.0 with hydrochloric acid before freezing (SPI-pH); both preparations were subsequently freeze-dried. The protein content of SPI was determined by the Lowry method [18]. At least three independent SPI-hydrolysis reactions were done.

### 2.2 Preparation of Emulsions

Oil-in-water emulsions were prepared at a 25:75 ratio (vol/vol) using the two SPI preparations at 1.0 mg/mL and 2.0 mg/mL in sodium phosphate buffer (0.01 M, pH 7). Emulsions were prepared at room temperature using an Ultra-Turrax homogenizer (IKA-Werke, Germany) with a S 25 N-10 G rotor at 20,000 rpm during 1 min.

### 2.3 Creaming Stability

All the emulsions were analyzed using a vertical optical analyzer Turbiscan Classic MA200 (Formulation, L'Union, France). The kinetics of emulsion creaming were determined by backscattering measures obtained every minute during 60 min, and by

measuring the reduction of mean values of backscattering ( $BS_{avg}$ ) in the range 10-20 mm ( $BS_{avg10-20}$ ) as a function of time [12]. The emulsions were prepared in triplicate and measurements of the stability were performed at least in triplicate.

### 2.4 Statistical Analysis

Data were statistically analyzed using analysis of variance (ANOVA) with  $\alpha = 0.05$  and by the least significant difference (LSD) test using Statgraphics Plus 7.0 software.

## 3. Results and Discussion

Creaming stability was analyzed by measuring the reduction of mean backscattering values in the lower portion (10-20 mm zone,  $BS_{avg10-20}$ ) of the tube as a function of time. Curves obtained for emulsions of 1 mg/mL protein concentration of SPI-F and SPI-pH are shown in Fig. 1. It was possible to observe that one of the curves is hyperbolic (SPI-F) while the other is sigmoidal (SPI-pH).

These results clearly show that emulsions prepared with both samples exhibit different kinetics behavior regarding creaming destabilization, and that it is necessary to develop kinetics to correctly characterize them.

Dagorn-Scaviner et al. [5] developed a method for determining the kinetics of creaming and flocculation

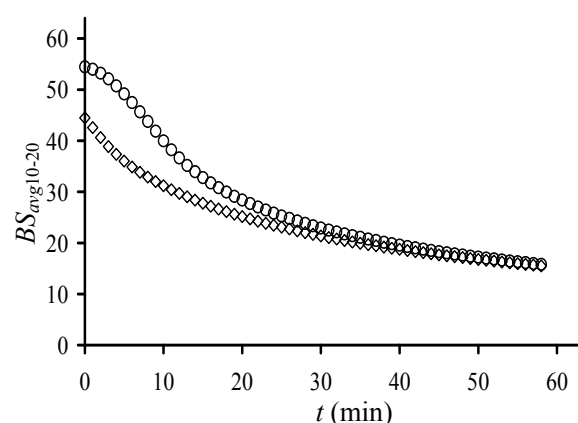


Fig. 1 Changes in mean backscattering values in the lower portion (10-20 mm zone,  $BS_{avg10-20}$ ) of the tube as a function of time for emulsions prepared with SPI-F ( $\diamond$ ) and SPI-pH ( $\circ$ ) (1 mg/mL protein concentration).

based on measuring the volume of the lower aqueous phase that appears after destabilization by creaming. These authors have shown that the experimental curve  $V_t$  vs.  $t$  that describes the variation of the aqueous phase as a function of time follows an exponential behavior:

$$V_e - V_f = V_e \cdot e^{-kt} \quad (1)$$

or in the form

$$\ln \left[ \frac{V_e}{(V_e - V_t)} \right] = kt \quad (2)$$

where,  $V_t$  is the volume of the aqueous phase at time  $t$ ,  $V_e$  is the volume of the aqueous phase in the equilibrium, and  $k$  is the rate constant of the process. These authors also showed that the plot  $\ln[V_e/(V_e - V_t)]$  vs.  $t$  presents successive segments that correspond to different stages with first order kinetics. Elizalde et al. [16] have experimentally shown that the destabilization of emulsions prepared with several protein solutions can be described by the following equation:

$$q(t) - q_0 = \frac{Q \cdot t}{(B + t)} \quad (3)$$

where  $q(t)$  is the moisture content at time  $t$ ,  $q_0$  is the moisture content of the recently prepared emulsion.  $Q$  is the maximal moisture content increment, and  $B$  is the time required to reach  $Q/2$ . In both, destabilization kinetics are followed by measuring the aqueous phase formed in the lower portion of the emulsion under creaming. The  $BS$  measure obtained with a vertical optical analyzer is linked to  $\Phi$  [19] and therefore also linked to  $(1 - \Phi)$ , i.e., the proportion of aqueous phase. In consequence, the hyperbolic curve shown in Fig. 1 may respond to an exponential equation and may follow first order kinetics as follows:

$$BS(t) = BS \cdot e^{-kt} + BS_f \quad (4)$$

where,  $BS(t)$  is the  $BS$  value at time  $t$ ,  $BS_f$  is the  $BS$  value at infinite time,  $BS$  is a value such that  $BS + BS_f = BS_i$  ( $BS_i$  is the initial  $BS$  value) and  $k$  the rate constant. Or it could follow second order kinetics with the expression:

$$BS(t) = \frac{BS \cdot B}{(B + t)} + BS_f \quad (5)$$

where,  $BS(t)$  is the  $BS$  value at time  $t$ ,  $B$  indicates the time required to reach  $BS_i/2$ ,  $BS_f$  corresponds to  $BS$  at infinite time, and  $BS$  corresponds to a value such that  $BS + BS_f = BS_i$ . Eqs. (4) and (5) were applied to the experimental data obtained with SPI-F at 1 mg/mL, which corresponded to a hyperbolic curve. Adjustments ( $r^2$ ) performed by using least square regression are shown in Table 1. Adjustment of the first order equation yielded a  $r^2 = 0.997$  and that of the second order equation yielded a  $r^2 = 0.9997$ , showing that the latter has a better fit. Since Eq. (5) represents second order kinetics, the rate constant  $k$  for  $BS$  reduction can be expressed as:

$$k = \frac{1}{(BS \cdot B)} \quad (6)$$

Palazolo et al. [12] proposed a very similar expression for  $k$ . Both expressions differ only in the  $BS$  value. While in Palazolo et al. [12] used the  $BS_i$  value, possibly assuming  $BS_f = 0$ , in the current study we used the  $BS$  as defined for Eq. (5). Therefore, Eq. (5) can be expressed as:

$$BS(t) = \frac{BS}{(BS \cdot k_h \cdot t + 1)} + BS_f \quad (7)$$

where  $k_h$  is the rate constant. For the SPI-pH curves (1 mg/mL), which showed a sigmoidal behaviour, the following equation is proposed:

$$BS(t) = \frac{BS \cdot C}{(C + t^2)} + BS_f \quad (8)$$

where,  $C$  is related to the time required for a reduction of  $BS_i$  to  $BS_i/2$  in the following manner:  $C^{1/2} = BS \cdot B$  and  $B$  values were estimated by least square regression. The adjustment of this equation yielded a  $r^2 = 0.9983$ , which was not as good as that of the hyperbolic curve. However, the adjustment to second order kinetics was discarded since it yielded  $r^2 = 0.9600$ . The application of the proposed kinetics (hereinafter referred as sigmoidal behaviour kinetics) to the experimental data of SPI-pH (1 mg/mL) was considered adequate. To obtain a rate constant expression for the process described by Eq. (8), an expression of the rate of reduction of  $BS$  by differentiation was derived:

Table 1  $r^2$  values for the different kinetics considered.

Protein	First order	Second order	Sigmoidal	Second order + sigmoidal
SPI-F (1 g/mL)	0.997 ± 0.001	0.9997 ± 0.0002		
SPI-F (2 mg/mL)		0.9977 ± 0.0001	0.9923 ± 0.0002	0.9998 ± 0.0002
SPI-pH (1 mg/mL)		0.9600 ± 0.0001	0.9984 ± 0.0001	0.9995 ± 0.0002
SPI-pH (2 mg/mL)			0.9997 ± 0.0002	0.9997 ± 0.0002

$$\frac{dBS}{dt} = \frac{(2 \cdot BS \cdot C \cdot t)}{(C + t^2)^2} \quad (9)$$

By expressing time as a function of  $BS$  from Eq. (8) and by replacing  $t$  for such expression in Eq. (9), the following equation was derived:

$$\frac{dBS}{dt} = \frac{2}{(BS \cdot C^{1/2})} \cdot (BS - BS_i)^{(2+1/2)} \cdot BS_i^{(2+1/2)} \quad (10)$$

where, the rate constant corresponding to the process is represented by:

$$k_s = \frac{2}{(BS \cdot C^{1/2})} \quad (11)$$

Eq. (8) can now be expressed as:

$$BS(t) = \frac{(2^2 \cdot BS)}{[2^2 + (k_s \cdot BS)^2 \cdot t^2]} + BS_f \quad (12)$$

and in the same fashion, Eq. (9) can be expressed as:

$$\frac{dBS}{dt} = \frac{(2^2 \cdot k_s^2 \cdot BS^3 \cdot t)}{[2^2 + (k_s \cdot BS)^2 \cdot t^2]^2} \quad (13)$$

Eqs. (7) and (12) would describe adequately the kinetics of variation of  $BS$  as a function of time in the interval considered. While  $BS$  changes correlate with the creaming process, the expression is empirical and only allows characterizing the process. In order to describe the process, the expression must have a physical meaning. As mentioned before,  $k$  is the rate constant of  $BS$  variation with time, and may be assumed as a rate constant describing the creaming process. But, some emulsions present a second order destabilization kinetics while others present the proposed sigmoidal behaviour kinetics. The rate of  $BS$  variation can be associated to the rate of the creaming process. Use Eq. (13) for the cases that follow the proposed sigmoidal behaviour kinetics, and the following expression is for those with second order kinetics.

$$\frac{dBS}{dt} = \frac{(k_h \cdot BS^2)}{(1 + k_h \cdot BS \cdot t)^2} \quad (14)$$

the rate of creaming of emulsions prepared with SPI-F and SPI-pH can be represented as shown in Fig. 2. For emulsions prepared with SPI-F at 1 mg/mL, the rate was maximal at the beginning and decreased thereafter. In contrast, for emulsions prepared with SPI-pH at 1 mg/mL, the creaming rate increased up to a maximum, which corresponded to the moment of the inflexion point in the  $BS_{avg(10-20)}$  vs.  $t$  curve, and then decreased in a fashion similar to emulsions prepared with SPI-F at 1 mg/mL. The ascending rate (creaming) of a spherical particle in a liquid is determined by the Stokes' law:

$$v = \frac{-2 \cdot g \cdot r^2 (\rho_1 - \rho_2)}{9 \cdot \eta_l} \quad (15)$$

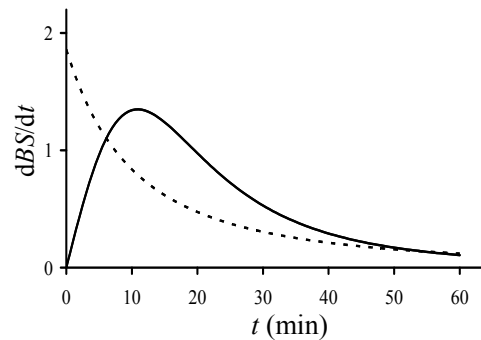
The  $BS$  values are a measure of concentration and mean size of the droplets in the emulsion as a function of the height of the measuring device [20].

$$BS \approx h/\lambda^* \quad (16)$$

and

$$\lambda^* = [2d/3\Phi \cdot (1 - g) \cdot Q_s] \quad (17)$$

where,  $h$  is the height of the detection zone,  $\lambda^*$  is mean free path length of photon transport,  $d$  is the diameter


 Fig. 2  $dBS/dt$  vs. time plot for emulsions prepared with SPI-F (---) and SPI-pH (—).

of the particle,  $\Phi$  is the oil volume fraction,  $g$  is an asymmetry factor, and  $Q_s$  is the efficiency factor of light scattering. Therefore,  $BS$  increases with  $d$  decreasing and  $\Phi$  increasing. The largest droplets are the ones with the highest creaming rate and, in consequence, would abandon the 10-20 mm zone of the tube more rapidly. As time elapses, the droplets that continue migrating in such zone exhibit progressively lower creaming rates in agreement with their size, such that it can be expected that the variation in creaming rate with time will behave as shown in Fig. 2 for SPI-F emulsions. For emulsions with a sigmoidal  $BS$  profile, in contrast, the behavior of the creaming rate would be reflecting that the movement of droplets, especially the larger ones, is hindered or delayed in the first interval of the creaming process, but this delaying effect would diminish with time, such that the creaming rate adopts a behavior similar to that of emulsions with hyperbolic pattern. Eq. (13) shows that  $k_s$  influence the magnitude of  $dBS/dt$ . Fig. 2 depicts two hypothetical  $dBS/dt$  vs.  $t$  curves (1 and 2) estimated using Eq. (13), where  $k_{s2} > 2k_{s1}$  and  $BS_1 = BS_2$ . It can be observed that the higher the  $k_s$  value is, the lower the time value ( $t_{vm}$ ) is corresponding to the maximal creaming rate.

The increment in the  $t_{vm}$  value would indicate a longer delaying effect of the creaming process. Therefore, it can be affirmed that the  $k_s$  value would be an indicator of such delaying effect and that a lower  $k_s$  would indicate a longer delaying effect. It can also be observed that once the maximum creaming rate was reached, since the magnitude and incidence of the delaying effect diminished, the creaming rate adopted a behavior similar to that of emulsions with hyperbolic pattern. The higher the  $k_s$  is, the faster the creaming rate decreases.

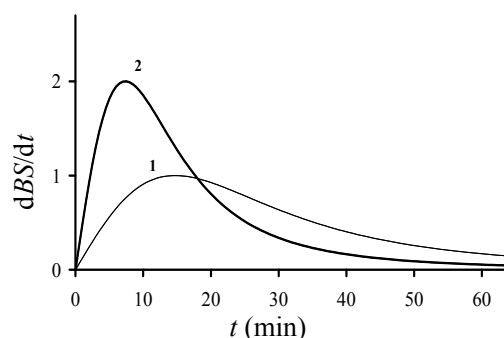
Strictly speaking, the Stokes' law can be used only to calculate the velocity of an isolated, rigid, spherical particle suspended in an ideal liquid of infinite extent [19]. In practice, there are often great differences

between the creaming rate predicted by the Stokes' law and those obtained experimentally in food emulsions, since many assumptions used in the deduction of the Stokes' law are not valid in the real situation. Several factors can determine a delay of the creaming rate predicted by the Stokes' law, as evidenced by the sigmoidal shape of the  $BS$  vs.  $t$  curve, including concentrated emulsions [21], charged droplets in the emulsion [22, 23], high effective density of the dispersed phase [24], polydispersed emulsions [25], size and structure of flocs within the emulsion [23], and high apparent viscosities at low shear speed of the dispersing phase [20, 23].

Fig. 3 shows the variation of  $BS_{avg10-20}$  with time for emulsions with a protein concentration of 2 mg/mL. It can be observed that curves for both SPI-F and SPI-pH have sigmoidal shape.

Eq. (12) was applied to the experimental data corresponding to SPI-pH (2 mg/mL) and SPI-F (2 mg/mL). Adjustments ( $r^2$ ) performed by least square regression are shown in Table 1. Emulsions prepared with SPI-pH yielded the best fit  $r^2 = 0.9997$ , while those prepared with SPI-F exhibited a  $r^2 = 0.9923$ . Fitting was not so good in the later case, and the correspondence between experimental values and those predicted with second order kinetics exhibited a  $r^2 = 0.9977$ , which is higher than that obtained for the sigmoidal behaviour kinetics.

As postulated before, for emulsions presenting a sigmoidal shape of  $BS$  vs.  $t$ , the magnitude and



**Fig. 3** Representation of two hypothetical  $dBS/dt$  vs.  $t$  curves (1 and 2) estimated by use of Eq. (14), where  $k_{s2} > 2k_{s1}$  and  $BS_1 = BS_2$ .

incidence of deviations from the Stokes' law are such that droplets cannot move during the first interval of the creaming process. As time elapses, the delaying effects will diminish so that the creaming rate reaches a similar value that the behaviour of emulsions with a hyperbolic pattern. The creaming rate can be represented as  $\Delta BS/\Delta t$  vs.  $t$ , as shown in Fig. 4. In emulsions prepared with SFI-pH 2 mg/mL, the creaming rate increased until a maximum, as expected, and then diminished in a way similar to that observed for emulsions with hyperbolic  $BS$  vs.  $t$  curves. Notably, the same behavior was observed for emulsions prepared with SFI-F 2 mg/mL. This would indicate that the sigmoidal behaviour kinetics for the kinetics will be more appropriate to describe the process than a second order one, although the adjustments ( $r^2$ ) seem to indicate the opposite. In view of the later results, in the case of emulsions with sigmoidal curves, it can be speculated that although many droplets have an impaired movement in the first stage of the creaming process because of the magnitude and incidence of deviations from the Stokes' law, other droplets may present simultaneously a behavior similar to that of droplets in emulsions with hyperbolic curves. As mentioned, this would occur simultaneously and not after that delaying processes have diminished. Therefore, for emulsions with sigmoidal  $BS$  curves, it was considered appropriate to develop a kinetics in which both types of behaviors are considered simultaneously:

$$BS(t) = \frac{BS_h}{(BS_h \cdot k_h \cdot t + 1)} + \frac{(2^2 \cdot BS_s)}{[2^2 + (k_s \cdot BS_s)^2 \cdot t^2]} \quad (18)$$

where,  $BS(t)$  is the  $BS$  value at time  $t$ ,  $k_h$  is the rate constant for  $BS$  variation as a function of time for second order kinetics,  $k_s$  is the rate constant for  $BS$  variation with time for sigmoidal behaviour kinetics, and  $BS_h$  and  $BS_s$  are amplitude measures for second order and sigmoidal kinetics, respectively, so that  $BS_h + BS_s = BS_i$ .

Eq. (18) was applied to the experimental data obtained in the present work, which yielded sigmoidal  $BS$  curves. The adjustments ( $r^2$ ) obtained by using least squares regression are shown in Table 2. The fit was very good in all cases and the correspondence was improved compared with previous kinetics. The values of  $k_h$ ,  $k_s$ ,  $BS_h$ ,  $BS_s$  as estimated by least squares regression are shown in Table 2.

Fig. 5 depicts the  $BS$  vs.  $t$  profile of the two destabilization processes considered in Eq. (18), as well as the curve for the total destabilization process. It can be seen that the destabilisation process corresponding to sigmoid kinetics is the first one to decrease with time for all the cases studied. For emulsion samples prepared

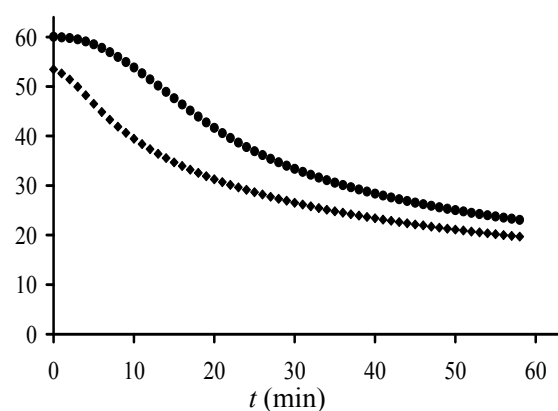


Fig. 4 Changes in mean backscattering values in the 10-20 mm zone ( $BS_{avg10-20}$ ) as a function of time in emulsions prepared with 2 mg/mL SPI-F (♦) and 2 mg/mL SPI-pH (●).

Table 2  $k_h$ ,  $k_s$ ,  $BS_h$  and  $BS_s$  values of emulsions prepared with dispersions of the studied proteins.

Protein	$k_h$	$k_s$	$BS_h$	$BS_s$
SPI-F (1 mg/mL)	$1.3 \times 10^{-3} \pm 0.5$		1.00*	
SPI-F (2 mg/mL)	$4.9 \times 10^{-4} \pm 0.5$	$1.7 \times 10^{-2} \pm 0.9$	$0.74 \pm 0.04$	$0.26 \pm 0.04$
SPI-pH (1 mg/mL)	$4.8 \times 10^{-4} \pm 0.3$	$5.4 \times 10^{-3} \pm 0.7$	$0.44 \pm 0.01$	$0.56 \pm 0.01$
SPI-pH (2 mg/mL)	$3.6 \times 10^{-5} \pm 0.3$	$2.3 \times 10^{-3} \pm 0.9$	$0.32 \pm 0.02$	$0.68 \pm 0.02$

\*HAPS-C protein (1 mg/mL) sigmoid curve model do not fit the data; the data correspond best with Eq. (5). Eq. (5) has a single term and only amplitude parameter thus its proportion is 1, informed in the column for  $BS_h$ .

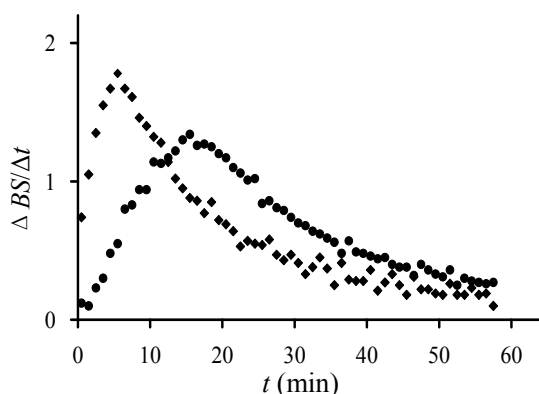


Fig. 5  $\Delta BS/\Delta t$  vs time plot for emulsions prepared with 2 mg/mL SPI-F (♦) and 2 mg/mL SPI-pH (●).

with SPI-pH (1 mg/mL and 2 mg/mL) destabilisation processes corresponding to sigmoidal kinetics (sigmoidal curve) predominates initially, but afterwards, second order kinetics prevail. On the contrary, for emulsion samples prepared with SPI-F (2 mg/mL) second order kinetics (hyperbolic curve) explain destabilisation process during the registered period.

This shows that not only  $k_h$  and  $k_s$  but also  $BS_h$  and  $BS_s$  are necessary to describe the destabilization process, since the latter allows to establish which is the contribution of each process to the whole destabilization phenomenon. For this purpose, it is more appropriate to determine the proportion of  $BS_h$  and  $BS_s$ . The fact that emulsions prepared with SPI-F (2 mg/mL) the second order kinetics contributes to destabilization process with 75% (Table 2) explains why the second order kinetic had a better fit than the sigmoidal behaviour kinetics (Table 1). Conversely, emulsions prepared with SPI-pH (2 mg/mL) presented a high contribution (68%) of the destabilization process with sigmoidal behaviour kinetics. Its  $k_h$  value with a lower order than that of SPI-F (2 mg/mL) explaining the absence of differences between the kinetics with sigmoidal behaviour kinetics and that combining sigmoidal—second order kinetics ( $r^2 = 0.9997$ , Table 1).

#### 4. Conclusions

Our results show that the behavior of the creaming

rate of emulsions with hyperbolic  $BS$  curves is such that the rate is maximal at the beginning of the process and diminishes later. In this case, deviations from the Stokes' law would be such that, although they affect the creaming rate of droplets, the expected tendency of larger droplets to cream faster is maintained. The second order kinetics describes the process adequately, as proposed by previous authors, and  $k_h$  could be equaled to a rate constant that describes the creaming process and its values would indicate the behavior of emulsions in terms of stability. For emulsions with a sigmoidal  $BS$  pattern, in contrast, the magnitude and incidence of deviations from the Stokes' law are such that droplet movement is impaired in the first interval of the creaming process. As time elapses, the delaying effects would diminish in magnitude and incidence so that the creaming rate would behave like that of emulsions with hyperbolic profile. In this case, kinetics with two terms, one of sigmoidal shape and one of second order, is adequate to describe the creaming process in contrast to kinetics previously reported in the literature. The  $k_h$  value has the same meaning as in the second order kinetics, and  $k_s$  indicates the delaying effect on the creaming rate.

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#### References

- [1] S. Damodaran, Protein-stabilized foams and emulsions, in: S. Damodaran, A. Paraf (Eds.), *Food Proteins and Their Applications*, Marcel Dekker, New York, 1997, pp. 57-110.
- [2] P.J. Halling, Protein-stabilized foam and emulsions, *Crit. Rev. Food Sci. Nutr.* 15 (2) (1981) 155-203.

- [3] K.N. Pearce, J.E. Kinsella, Emulsifying properties of proteins: Evaluation of a turbidimetric technique, *J. Agric. Food Chem.* 26 (3) (1978) 716-723.
- [4] M. Shimizu, T. Takahashi, S. Kaminogawa, K. Yamauchi, Adsorption onto an oil surface and emulsifying properties of bovine  $\alpha_{s1}$ -casein in relation to its molecular structure, *J. Agric. Food Chem.* 31 (6) (1983) 1214-1228.
- [5] C. Dagorn-Scaviner, J. Gueguen, J. Lefevre, Emulsifying properties of pea globulins as related to their adsorption behaviours, *J. Food Sci.* 52 (2) (1987) 335-341.
- [6] E. Keowmaneechai, D.J. McClements, Influence of EDTA and citrate on physicochemical properties of whey protein-stabilized oil-in-water emulsions containing  $\text{CaCl}_2$ , *J. Agric. Food Chem.* 50 (24) (2002) 7145-7153.
- [7] J.D. Firebaugh, C.R. Daubert, Emulsifying and foaming properties of a derivatized whey protein ingredient, *J. Inter. Food Properties* 8 (2) (2005) 243-253.
- [8] B. Latreille, P. Paquin, Evaluation of emulsion stability by centrifugation with conductivity measurements, *J. Food Sci.* 55 (6) (1990) 1666-1668.
- [9] O. Mengual, G. Meunier, I. Cayré, K. Puech, P. Snabre, Turbiscan MA 2000: Multiple light scattering measurement for concentrated emulsion and suspension instability analysis, *Talanta* 50 (2) (1999) 445-456.
- [10] R. Chanamai, D.J. McClements, Impact of weighting agents and sucrose on gravitational separation of beverage emulsions, *J. Agric. Food Chem.* 48 (11) (2000) 5561-5565.
- [11] L.G. Pan, M.C. Tomás, M.C. Añón, Effect of sunflower lecithins on the stability of water-in-oil and oil-in-water emulsions, *J. Surfactants Deterg.* 5 (2) (2002) 135-143.
- [12] G.G. Palazolo, D.A. Sorgentini, J.R. Wagner, Emulsifying properties and surface behavior of native and denatured whey soy proteins in comparison with other proteins: Creaming stability of oil-in water emulsions, *J. Am. Oil Chem. Soc.* 81 (7) (2004) 625-632.
- [13] C.C. Sánchez, J.M. Rodríguez Patino, Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration in solution, *Food Hydrocolloids* 19 (3) (2005) 407-416.
- [14] S. González-Pérez, G.A. van Koningsveld, J.M. Vereijken, K.B. Merck, H. Gruppen, G.J. Voragen, Emulsion properties of sunflower (*Helianthus annuus*) proteins, *J. Agric. Food Chem.* 53 (6) (2005) 2261-2267.
- [15] A.R. Taherian, P. Fustier, H.S. Ramaswamy, Steady and dynamic shear rheological properties, and stability of non-flocculated and flocculated beverage cloud emulsions, *J. Inter. Food Properties* 11 (1) (2008) 24-43.
- [16] B.E. Elizalde, A.M.R. Pilosof, G.B. Bartholomai, Kinetics of demulsification of food protein-stabilized oil-in-water emulsions, *J. Am. Oil Chem. Soc.* 66 (10) (1989) 1454-1458.
- [17] S. Petrucci, M.C. Anon, Relationship between the method of obtention and the structural and functional properties of soy protein isolates—1. Structural and hydration properties, *J. Agric. Food Chem.* 42 (10) (1994) 2161-2169.
- [18] O.H. Lowry, H. Rosebroug, A. Lewis, R.J. Randall, Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1) (1951) 265-275.
- [19] E. Dickinson, An Introduction to Food Colloids, Science Publications, Oxford, 1992, p. 30.
- [20] B. Abismail, J.P. Canselier, A.M. Wilhelm, H. Delmas, C. Gourdon, Emulsification processes: On-line study by multiple light scattering measurements, *Ultrasonics Sonochem.* 7 (4) (2000) 187-192.
- [21] V.J. Pinfield, E. Dickinson, M.J.W. Povey, Modelling of concentration profiles and ultrasound velocity profiles in a creaming emulsion: Importance of scattering effects, *J. Colloid Interf. Sci.* 166 (2) (1994) 363-374.
- [22] E. Dickinson, G. Stainsby, The oil-water interface and emulsion stability, in: E. Dickinson, G. Stainsby (Eds.), *Colloids in Food*, Applied Science Publishers, London, 1996, p. 107.
- [23] P. Walstra, Emulsion stability, in: P. Becher (Ed.), *Encyclopedia of Emulsion Technology*, Marcel Dekker, New York, United States, 1996, p. 1.
- [24] C.T. Tan, Beverage emulsion, in: S. Friberg, K. Larsson (Eds.), *Food Emulsion*, Marcel Dekker Inc., New York, 1990, p. 445.
- [25] S. Dukhin, J. Sjöblom, Kinetics of brownian and gravitational coagulation in dilute emulsions, in: J. Sjöblom (Ed.), *Emulsions and Emulsions Stability*, Marcel Dekker, New York, 1996, p. 41.